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TITLE: The Discovery of New Antimicrotubule Agents from Hawaiian, Polynesian, and Asian Ethnobotanical Sources

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**13. ABSTRACT** ***(Maximum 200 Words)***

An extract library of 790 extracts of higher plants has been prepared. Plants were selected for extraction based on ethnobotanical information indicating use in cancer therapy in the traditional medical systems of societies of the Pacific Rim.

Extracts were tested for cytotoxicity in drug-sensitive and drug-resistant breast cancer cell lines and in mechanism-based assays for activity against the cytoskeleton, specifically microtubules and microfilaments.

Selected extracts that showed activity in any of the assays were investigated by bioassay-guided fractionation to isolate the active principles and to establish their chemical structures.

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## Introduction

Great strides are being made in broadening the arsenal of available chemotherapeutic arsenal for the treatment of breast cancer, as was obvious at the 2002 Era of Hope meeting. Among the new agents biologics such as herceptin appear to have the most immediate benefit. What appears to be sometimes overlooked in this era of non-small molecule therapeutics, is the fact that these new strategies, like the old ones, only benefit a fraction of the population of breast cancer patients who require new and better drugs. Drug discovery programs directed at classical targets will therefore still be able to contribute to the overall goal of eradicating breast cancer for some time to come. Of particular interest are agents with better efficacy, i.e. improved activity vs. toxicity profile, and those with activity against multi-drug resistant solid tumors. The clinical importance of the latter group may not have been apparent when the screening programs were initiated that led to the discovery of the agents now in clinical use.

With funding from the Department of Defense Breast Cancer Research Program we initiated a screening program directed at the discovery of natural products with activity against the cytoskeleton, specifically microtubules and microfilaments, as indicated by mechanism-based assays. As biological source materials we proposed to use ethnobotanically indicated plants of the Pacific Rim, broadly speaking, which had been introduced to Hawai'i.

## Summary of the Research

### 1 Plant collection and extract preparation

As summarized in the Year 3 report, in the course of the research we prepared an extract library of 790 extracts from 395 plant samples, which were derived from 309 plant species. We have thereby exceeded by a considerable margin the number of extract (600 extracts from 300 species) originally proposed (Task 1). Where the ethnobotanical literature indicated use of different plant parts of a given species, we collected and extracted these materials separately. Hence the number of extracts exceeded that of the number of plant species investigated. Each sample yielded a lipophilic (dichloromethane/isopropanol (2:1)) and a hydrophilic (70% aqu. ethanol) extract. Aliquots of these were submitted for testing and the remaining material has been deposited in our extract library as dried material that is stored at –20°C.

### 2 Testing for Biological Activity

During the research under this award the scope of the testing to be undertaken was expanded from screening for antimicrotubule activity only, to also include the identification of extracts with antimicrofilament activity and to cytotoxic extracts. In the latter group special emphasis was placed on those that displayed cytotoxic effects on cells of the multi-drug resistant phenotype as result of overexpression of the p-glycoprotein drug-efflux pump. A database of all cytotoxicity screening data can be found in the Appendix for the Year 3 report.

## 2.1 Aqueous extracts

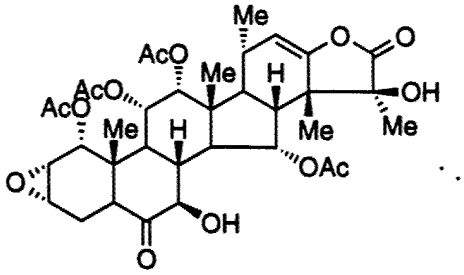
While it has been reported that screening of the NCI repository of extracts has identified several aqueous extracts with biological activity,[1] this was not the case in our extract library. Thus, neither in the cytoskeletal nor in the cytotoxicity assays did we identify an aqueous extract with biological activity.

## 2.2 Organic Extracts

As summarized in the Year 3 report, screening of the organic crude extracts of 395 plant samples yielded ten extracts with activity in the indirect immunofluorescence assay that was employed to detect anticytoskeletal effects. Two showed a taxol-like effect of microtubule stabilization and eight extracts displayed a vinblastine-like microtubule depolymerizing effect.

### 2.2.1 Extracts with Taxol-like effects

CC097L and CC283L were both extracts of species of the genus *Tacca* (Taccaceae). The active principle of extract CC097 from *Tacca chantrieri* was isolated by bioassay guided fractionation and identified as taccalonolide A **1**, an unusual heptacyclic steroid (Fig. 1). At the time this was only the material with this mode of action and to this day remains only the second one from the plant kingdom. The material had been isolated earlier from another *Tacca* species based on a cytotoxicity screen against L1210 cells.[2]




**1**

**Fig. 1.** Structure of taccalonolide **1**.

Because of scarcity of plant material, we did not pursue the fractionation of *Tacca palmata*, the species that gave rise to extract CC283L. It seems reasonable that the activity is caused by taccalonolide A or a very similar compound, given that CC097L and CC283L were extracts from plants of the same genus.

The discovery of taccalonolide by means of a mechanism-based screening program is the single most important discovery made under this award. It shows that a small team with modest means can compete with industrial screening programs that have, in comparison, limitless resources available. There are anecdotal reports at scientific meetings that several industrial screening programs came up dry with regard to hits with taxol-like activity after screening literally tens of thousands of extracts.

Taccalonolide A is of interest for mainly two reasons. First, it has activity against drug-resistant cells overexpressing the p170 drug-efflux pump and drug-sensitive cells, in difference to the clinically used microtubule poison Taxol. Since the development of multi-drug resistance during the treatment of solid tumors is a common, clinically encountered problem, leads that can circumvent this mechanism are of interest. Second, the structure of taccalonolide can presumably be simplified as a result of a study of the structure-activity relationships of the natural product, work that is ongoing. As a result the considerable expertise available in the area of steroid chemistry can be brought to bear on the development of an analog that is more amenable to synthesis than the structure of **1**. One may wish to contrast this with the experience with discodermolide and eleutherobine, two marine natural products with taxol-like activity, which are not accessible by isolation from the natural source or by synthesis on scales that would allow clinical development.

### 2.2.2 Extracts with vinblastine-like effects

For reasons that are not altogether clear, this type of activity is much more common among microtubule poisons isolated from fungi, bacteria, marine organisms and higher plants. We were therefore not surprised to find a higher number of hits with a vinblastine-like effect in our library of organic extracts. As summarized in the Year 3 report, eight extracts showed this type of activity during the initial screening.
We have found that the microtubule-disrupting activity in those extracts that were not particularly potent proved hard to reproduce on recollection. Thus extracts displaying +++ activity (≈ 75% microtubule loss) at 50μg/mL, e.g. CC106L, CC 133L, CC144L, and CC147L could not be confirmed upon repeated recollection as reported in the Year 3 report. We made a similar experience with CC337L from *Hibiscus rosa-sinensis*, which was fractionated and showed spurious activity in fractions and in assays of crude extracts after recollections. Bioassay-guided fractionation eventually led us to a fraction containing free fatty acids. Extracts from CC308L, bark of *Premna sp.*, could not be investigated further because a bark sample of sufficient size could not be obtained from Lyons Arboretum, the only location of a *Premna* tree in the State of Hawaii known to us. Extract CC309L, from leaves of *Premna sp.* did not show a vinca-like effect on repeated testing.

The reasons for the non-reproducibility of the vinca-like antimicrotubule activity are presumably several and deserve comment. First, we chose deliberately not to recollect exclusively from the plant that had given rise to the original specimen, but also from other individuals. This was done in the expectation that one individual plant could not yield sufficient plant material for preparative extraction and bioassay-guided isolation. Second, we were also concerned to pick up anticytoskeletal activity from a metabolite produced by a fungal sapprophyte rather than by the plant itself we were collecting. By collecting samples from different individuals we could reduce the risk of this happening. Picking up trace amounts of a potent agent of microbial origin while working on a plant-derived extract is a serious concern. This problem will show up particularly in weakly active crude samples because of the large difference in mass that the sapprophyte and the plant contribute to the overall sample mass. Specific examples include maytansine, an

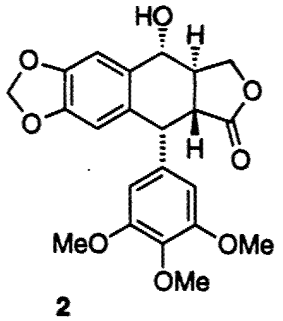
antimicrotubule agent commonly found in *Celastraceae, Rhamnacecae* and *Euphorbiaceae.*[3,4,5,6,7] Maytansinoids, which differ in the structure of one side chain, are also produced by *Actinomycetes*, specifically *Actinosynnema pretiosum* var. *pretiosum.*[8,9] A second example is the production of taxol by endophytes of plants other than *Taxus sp..*[10,11] Third, the indirect immunofluorescence assay may not be as robust as we would like when crude extracts are subjected to the procedure. The experienced microscopist can sometimes tell that a vinblastine-like effect is "secondary". This means that the cytoskeleton is affected, but only as a result of a primary toxic effect that hits a different target or of non-specific cytotoxicity that is not so strong that the cells fragment. The case of CC337L, which yielded a fatty acid may be used to illustrate the point. It is obvious that free fatty acids, which are known to be weak cytotoxins, have no primary effect on the cytoskeleton. Thus, either the bioassay-guided fractionation that results in the isolation of such material was misled by a "secondary" effect or the real active ingredient with bona-fide antimicrotubule effect was present only in trace amounts and was lost upon repeated chromatography. In the case of CC337L we tend to put more faith in the former explanation since two independent fractionations by different students came to the same conclusion. The expected enrichment of biological activity, i.e. an increase in potency as the purification progresses was not evident. This best indication of a successful purification is not always easily discernable from one purification step to the next in a multi-step procedure. This problem arises especially while using semi-quantitative assay procedures, such as the immunofluorescence assay for cytoskeleton poisons. However, the enrichment is nonetheless evident over the course of a successful multi-step purification scheme, as in the case of *H. ovigera* or *T. chantrieri.*

A cell-free tubulin (de)polymerization assay similar to the one that led to the discovery of the epothilones could be used for confirmation of a hit in the primary cytoskeleton screen.[12] However, we deliberately chose not follow this route. The principal reason for this decision was that the experience with the cell-based assay suggests that A-10 cells actively enrich the cell lumen with small molecules added to the medium. This results in higher intracellular concentrations of small molecules added to the cells than the nominal concentration in the well of the 96-well plate would suggest. Although one would gain in the cell-free assay specificity of detection of the desired cell biological effect, e.g. microtubule destabilization in the present case, the sensitivity would be significantly reduced. Unfortunately, this reduced sensitivity cannot be compensated for to the degree necessary by adding more extract because of nonspecific binding etc.. Also, one cannot simply dismiss out of hand all of the extracts with weak vinca-like activity either, because one is dealing with very crude extracts. The potency of the biological effect is obviously a function of the concentration of the active component in the extract, which may happen to be low. One is therefore stuck with doing the dereplication the hard way by bioassay-guided fractionation.

Extract CC001L of *Rumex cripsus* was the only exception to the observation that only weakly active extracts were irreproducible. Crude extracts from some collections of this species showed activity at 100-fold dilutions. Nonetheless, we were not able to focus the activity within a narrow range of fractions as the bioassay-guided separation progressed. This observation raised our suspicions that we were dealing with an artifact. As

mentioned above in connection with taccalonolide **1**, it is most unusual that only one species of a genus elaborates a given molecule of potent biological activity. We therefore also acquired plant material from two other species of the genus *Rumex*, which both tested negative in the mechanism-based and the cytotoxicity screen at 50μg/mL. The lead was therefore abandoned.

Extract CC142L of *Hernandia ovigera* was extremely potent and the activity was traced to podophyllotoxin **2**, a known potent antimicrotubule agent (Fig. 2). We had missed the significance of reports on the occurrence of the compound[13] in *Hernandia* because the semisynthetic glucoside derivative of podophyllotoxin, etoposide, is a topoisomerase inhibitor.[14] We were unaware of this change in molecular target when we were establishing the list of known plant-derived antimicrotubule agents to watch for during literature dereplication.

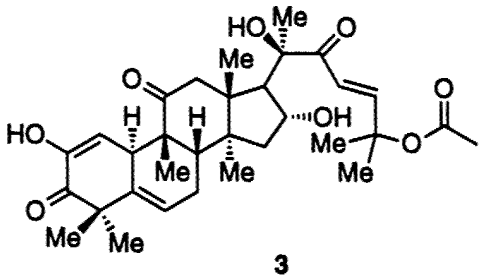



**2**

**Fig. 2.** The structure of podophylotoxin.

Antimicrofilament activity

As part of the screening process we had also screened for extracts with antimicrofilament activity as reported in the Year 1 report. Two extracts were found to be active CC103L and CC278L. Only the activity in CC278L, an extract from *Bacopa monnieri*, was reproducible. Although several reports on the isolation of biologically active compounds from this species were found during literature searches, the compounds described, mostly triterpene glycosides, were deemed to be unlikely to be responsible. Bioassay–guided fractionation and structure elucidation (see Appendix) led to the isolation of cucurbitacin E **3** (Fig. 3), a compound not previously described from *B. monnieri*, but well known from other sources and known to be an antimicrofilament agent.



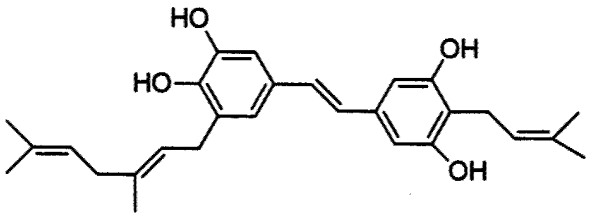

**3**

**Fig. 3.** The structure of cucurbitacin E.

### 2.2.3 Cytotoxicity Screen

The screening of the extract library for cytotoxins was directed at those extracts that showed comparable activity against p-glycoprotein overexpressing cells and those that are not multi-drug resistant. Extracts CC121L, CC168L, CC200L, CC329L and CC363L were selected for follow-up based on potency and/or absence of literature data on compounds isolated from the species that gave rise to the extracts.

Mappain (**4**)
The bioassay–guided fractionation of CC121L, an extract of *Macaranga mappa*, led to the isolation of mappain **4** (Fig. 4), a prenylated stilbene, the structure of which was published (see Appendix for reprint). The compound displays an $IC_{50}$ of 4 µg/mL and 3 µg/mL against drug-resistant and drug-sensitive cells, respectively. Investigations into the mechanism of action of mappain were initiated under this award and some preliminary data were included in the Year 3 report. These studies are now being continued in Dr. Mooberry's lab under different funding.




**4**

**Fig. 4.** The structure of mappain **4**.

(+)-Falcarindiol (**5**) and related compounds
The bioassay-guided fractionation of CC329L from *Tetraplasandra hawaiiensis*, a Hawaiian endemic plant, yielded a mixture of several polyacetylene oxylipins of which falcarindiol was the major constituent. Minor components were identified as crithmumdiol **6** and an epoxidized derivative **7** of falcarindiol (Fig. 5).

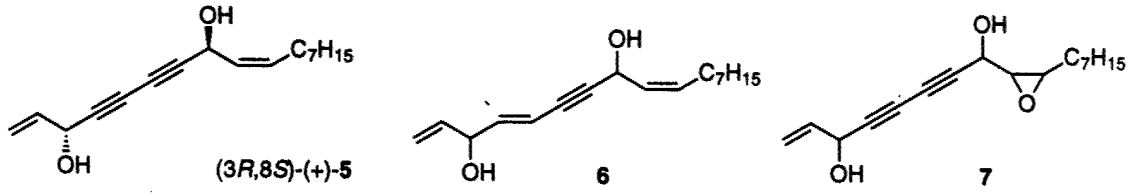



Fig. 5. Structures of (+)-falcarindiol **5**, crithmumdiol **6** and an unnamed oxylipin **7** from *T. hawiiensis*.

Falcarindiol is a compound of potent and diverse biological activity and has therefore been isolated from a variety of sources. While we were determining the total structure of our isolate, we became aware of contradictions in the literature concerning the

stereochemistry of **5**. We therefore established a procedure for the degradation of falcarindiol to determine the stereochemistry of **5** from *T. hawaiiensis* by an independent, non-empirical approach. This method has been submitted for publication to Org. Lett. (see Appendix). With the absolute stereochemistry of the parent oxylipin **5** defined, we are now in the position to calibrate the experimentally much more simple empirical methods of stereochemical analysis, such as the advanced Mosher method. Once we have identified a suitable method, we will complete the stereochemical analysis of **6** and **7**, which should result in another publication. At the present time only the gross structure, I.e. the atom connectivity, is known for compounds **6** and **7**.

Michelalide (**8**)

Extract CC168L from *Michelia champaca* was also selected for bioassay-guided fractionation and structure elucidation to yield the known sesquiterpene lactone michelalide (8-acetoxy-parthenolide) **8**. This material had been isolated from other *Michelia* species before.[15,16] Detailed cytotoxicity data for purified michelalide were not collected since sesquiterpene lactones such as michelalide are broad-spectrum alkylating agents which have to be regarded as nuisance compounds.

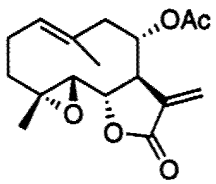



**Fig. 6.** The structure of michelalide 8.

Extracts CC200L and CC363L

These two extracts from *Pittosporum tobira* and *Ardisia solanacea*, respectively, were worked on repeatedly by different coworkers using a cytotoxicity-guided fractionation scheme. This work yielded "active" fractions containing fatty acids or materials that looked like fatty acids by $^1$H NMR. However, a tractable, non-trivial compound was never obtained and work on these extracts was abandoned.

## 3 Mechanism of Action Studies on Taccalonolide and Mappain

The results of these studies performed under the present award have been documented in the Year 3 report. With Dr. Mooberry's departure from the Cancer Research Center of Hawaii, the work was continued under funding from a different source. Two manuscripts reporting the findings to date on these two compounds are in preparation.

## Key Research Accomplishments

- Identification of 87 plant extracts that are cytotoxic to MDA-MB-435 cells with at least 50% inhibition of cell proliferation at a concentration of 20μg/ml
- Discovery of a new cytotoxic, microtubule-stabilizing agent, taccalonolide A, from *Tacca chantrieri* with activity against breast cancer cell lines
- Discovery of a new cytotoxic agent, mappain, from *Macaranga mappa*

## Reportable Outcomes

**Publications:**
Published and submitted (see Appendix):

van der Kaaden, J.D., Hemscheidt, T. and Mooberry, S.L. Mappain, a new cytotoxic stilbene from *Macaranga mappa J. Nat. Prod.* **2000**, *64*, 103-105.

Ratnayake, A. S.; Hemscheidt, T. Olefin Cross-metathesis as a Tool in Natural Products Degradation. The Stereochemistry of (+)-Falcarindiol. *Org. Lett.* **2002**, *4*, XXXX

In preparation:

Mooberry, S. L.; Tinley, T. L.; Hernandez, A. H.; Tien, G.; Hemscheidt, T. K. Taccalonolide A, A Plant-Derived Steroid with Taxol-like Microtubule-Stabilizing Activity. To be submitted to *Cancer Research.* (Data summarized in Year 3 report)

Ratnayake, A. S.; Mooberry, S.L.; Hemscheidt, T. Polyacetylene Oxylipins from *Tetraplasandra hawaiiensis*. To be submitted to *Nat. Prod. Lett.*

**Conference Abstracts**:

Mooberry, S, L. Discovery of a New Microtubule-Stabilizing Agent from a Tropical Plant. Proceedings of the American Association for Cancer Research **2000**, *41*, 553.

Mooberry, S. L. The Discovery of a New Microtubule-stabilizing Agent. Era of Hope, Department of Defense Breast Cancer Research Program Meeting Proceedings **2000**, 745.

Tinley, T. L; Mooberry, S. L. Taccalonolide A: A New Microtubule-Stabilizing Natural Product. French-American Colloquium on the Cytoskeleton and Human Disease. April 17-20, 2001. Marseille, France. Poster PB21.

**Patent Application:**

Hemscheidt, T. K.; Mooberry, S. L. Taccalonolide microtubule stabilizing agents. PCT/ US00/13795 May 18, 2000 (see Appendix Year 3 report)

**Theses:**

Isolation and Identification of Bioactive Metabolites from Marine Microorganisms and a Terrestrial Plant. M.Sc. Thesis, Peter C. Chow, May 2002.

Isolation, Structure Elucidation and Synthesis of Bioactive Metabolites from Plants and Fungi, projected Ph. D. thesis of Anokha S. Ratnayake, to be submitted Summer '03.

**Grant Applications (Funded):**

The Molecular Mechanisms of Action of a New Taxol-Like Microtubule-Stabilizing Agent. (DAMD 17-00-1-0284) Susan L. Mooberry, PI.

This proposal is a direct result of our work in the current grant with the discovery of taccalonolide A as a new Taxol-like agent.

Semi-synthesis and In Vitro Anticancer Evaluation of Derivatives of a New Microtubule Poison with a Taxol-like mechanism. (DAMD17-00-1-0-282) Thomas K. Hemscheidt, P.I.

This proposal is a direct result of our work in the current grant with the discovery of taccalonolide A as a new Taxol-like agent.

**Employment and Educational Opportunities:**

Peter C. Chow, M. Sc., now works for the Food and Drug Administration Pacific Northwest Regional Laboratory in Bothell, WA.

Matthew Vierra now works as a laboratory technician at the Cancer Research Center of Hawaii.

Janice Loy got her start in research in my laboratory and is now a first year graduate student at the Department of Chemistry UC Irvine.

**Conclusions**

The goal of the proposed research was the discovery of new antimicrotubule agents as lead structures for the development of new therapeutics that might find use in the treatment of breast cancer. To this end we employed a mechanism-based screen that had

not been used extensively for the screening of extracts from higher plants. The plants were selected for extraction on the basis of ethnobotanical information available in the literature and their availability in the State of Hawaii.

It is our current view that it is doubtful that the ethnobotanical information helps to prioritize plants for investigation in any meaningful way. Ethnobotanical information is generally too vague and does not follow western disease nomenclature. It appears in retrospect that we might have obtained a higher hit rate of interesting extracts to follow up on if we had undertaken a comprehensive rather than a selective study of plant species available in this State.

As is often the case, the mechanism-based assay employed for the identification of antimicrotubule agents in crude extracts from biological materials gave a fair number of false positives. However, this problem does not seem to be any more severe than in other assays or for identification of leads in combinatorial chemistry-derived libraries, which are only nominally more pure. False positives are simply a corollary of screening and dereplication requires a great deal of effort, irrespective of the source of the small molecule..

The discovery of taccalonolide A as the second plant-derived microtubule-stabilizing agent with a taxol-like mechanism is significant. Only seven molecules of this mechanism of action are known in the open literature. The urgency with which these leads are being pursued both in industry and in academia highlights the importance that the scientific community places on this mechanism of action and its clinical potential.

The further characterization of the biological activity is being actively pursued in our laboratories at the present time and these studies will continue over the next few years. This includes both more detailed mechanism of action studies, structure-activity relationships and in vivo evaluation. It is fair to say that without funding from the DoD Breast Cancer Research Program none of this work would have been undertaken. More conventional funding agencies would have found the odds of a discovery too low to consider funding such a program.

It is important to realize, however, that the first important step that may lead to a new drug has been taken. We have found a molecule that hits a therapeutically important and clinically validated target. Preliminary data suggest that there are some differences in the mechanism of action between taccalonolide A and some of the other microtubule stabilizers. Thus, taccalonolide is not "just another antimicrotubule agent". Although its clinical potential is unknown at present and an enormous amount of work needs to be done, the discovery of this entity is quite significant.

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## APPENDIX LIST

| | | |
|---|---|---|
| 1. | Reprint of *J. Nat. Prod.* **2001**, *64*, 103-105. | 3 Pages |
| 2. | Reprint of *Organic Letters* **2002**, *4*, XXXX and Supplementary Information | 10 Pages |
| 3. | Summary of NMR data for Cucurbitacin E from *Bacopa monnieri* | 1 Page |
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# Mappain, a New Cytotoxic Prenylated Stilbene from *Macaranga mappa*

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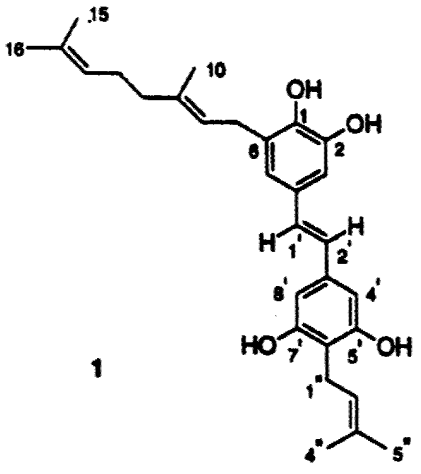
A new prenylated stilbene, mappain (**1**), was isolated from leaves of *Macaranga mappa* by bioassay-guided fractionation. The structure was established by application of spectroscopic methods. Mappain is cytotoxic but it appears to be a poor substrate for P-glycoprotein-mediated transport because it is equally potent and effective against the drug-sensitive SK-OV-3 and drug-resistant SKVLB-1 ovarian cancer cell lines, exhibiting an $IC_{50}$ value of 1.3 $\mu$M in both cases.

In the course of an investigation to identify new anticancer lead compounds from Asian botanical sources, we collected *Macaranga mappa* (L.) Muell. Arg. (Euphorbiaceae) (from the Lyon Arboretum of the University of Hawaii. The lipophilic crude extract of leaves of this species displayed significant cytotoxicity against both drug-resistant (SKVLB-1) and drug-sensitive (SK-OV-3) ovarian cancer cell lines ($IC_{50}$ of 3.5 $\mu$g/mL). A literature review indicated that the genus *Macaranga* with its 300 species[1] has not been investigated phytochemically in great detail. A prenylated flavonol[2] and a hexahydroxanthene derivative, vedelianin,[3] were isolated from *M. vedeliana* from New Caledonia. Prenylated flavanones[4] have been reported from *M. pleiostemona*, and chromenoflavones have been isolated form *M. indica*.[5] Consequently, the *M. mappa* extract was selected for bioassay-directed fractionation, which yielded mappain (**1**) as the active constituent. While this work was in progress, two publications from the Laboratory of Drug Discovery Research and Development of the NCI appeared describing the isolation and structure elucidation of the related schweinfurthins[6] and of a prenylated flavone[7] from *M. schweinfurthii*. A total synthesis of schweinfurthin C has been reported recently.[8]




**1**

Freeze-dried leaf material was extracted with $CH_2Cl_2$−*i*-PrOH, and after evaporation the extract was subjected to a modified Kupchan partition scheme. The $CHCl_3$-soluble fraction was cytotoxic and was subjected to flash chromatography on Si gel. Final purification was achieved by gel filtration over LH-20 followed by flash chromatography on Si gel. Mappain (**1**) was isolated as a beige powder in 0.026% yield of the fresh weight.

The HREIMS of **1** showed a molecular ion of *m/z* 448 (found 448.2596), which matched a molecular formula of $C_{29}H_{36}O_4$(Δ 1.8 mmu). The $^{13}C$ NMR spectrum of **1** displayed 27 signals comprising five methyl, four methylene, eight methine, and 12 quaternary carbon atoms based on analysis of the HMQC spectrum. The difference between the molecular formula deduced by mass spectrometry and the number of carbon atoms visible in the $^{13}C$ NMR spectrum suggested the presence of an element of symmetry in one part of the structure of **1**. This was confirmed by the analysis of the $^1H$ NMR spectrum in $CDCl_3$. Thus, the presence of one two-proton singlet at 6.53 ppm and one two-proton singlet for exchangeable hydrogen atoms at 5.13 ppm suggested that this element of symmetry was present in an aromatic ring in the form of a 1.3 bisphenol (ring B). Further analysis of the downfield portion of the $^1H$ NMR spectrum indicated two additional aromatic signals in the form of a pair of one-proton doublets ($J$ = 1.8 Hz) at 6.78 and 6.93 ppm, respectively, suggesting the meta-orientation of two protons on an aromatic ring, as well as a pair of one-proton doublets ($J$ = 16.1 Hz) at 6.74/6.83 ppm. This latter system was indicative of a trans double bond linking two aromatic rings and suggested that **1** was a substituted stilbene, which was corroborated by the UV/vis spectrum [$\lambda_{max}$ 330 (log $\epsilon$ 3.55) nm, MeOH]. Two of the remaining three substituents on ring A of this stilbene were identified in the form of two exchangeable protons resonating at 5.38 and 5.48 ppm, respectively, suggesting the presence of two phenolic groups. This analysis left one position on each of the two aromatic rings of the stilbene system for the attachment of the remaining structural elements, which were composed of 15 carbon atoms and 26 hydrogen atoms.

Analysis of the remaining signals in the $^1H$ NMR spectrum of **1** indicated the presence of five methyl singlets between 1.60 and 1.83 ppm, a four-proton multiplet at 2.10 ppm, two closely spaced two-proton doublets ($J$ = 7.1 Hz) between 3.38 and 3.42 ppm, as well as three olefinic methine multiplets between 5.07 and 5.34 ppm. These resonances and the remaining 15 carbon resonances were assigned to a prenyl and a geranyl group, respectively,

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mainly by straightforward analysis of HMBC and COSY spectra.

Placement of the substituents on the aromatic rings of **1** was accomplished by analysis of the HMBC spectrum. Thus, the two-proton singlet at 6.53 ppm for the aromatic protons on ring B showed HMBC correlations to both of the carbon atoms of the styrene double bond. This supported the placement of these protons at C-4′/C-8′ rather than at C-5′/C-7′, as would be expected also from biogenetic considerations. The resonance at 112.9 ppm is assigned to the nonprotonated carbon atom C-6′, based on correlations to the two-proton singlet at 6.53 ppm (H-4′, H-8′) as well as to the two-proton singlet at 5.13 ppm for the two exchangeable phenolic protons (OH-5′, OH-7′). An additional correlation of the resonance due to C-6′ to the two-proton doublet at 3.42 ppm (H-1″) identified C-6′ as the site of attachment of the prenyl group.

The placement of the two aromatic protons of ring A in the ortho position relative to the styrene double bond was supported by HMBC correlations from the proton resonance at 6.83 ppm due to the proximal styryl proton to both C-3 and C-5. Placement of the phenolic substituents at C-1 and C-2 was based on HMBC correlations of H-3 to C-1 and C-2, whereas H-5 correlated only to C-1. Attachment of the geranyl group via the two-proton doublet at 3.38 ppm to ring A was based on HMBC correlations of the carbon atoms C-6 (127.4 ppm), C-5, and C-1 to the proton signal at 3.38 ppm.

Mappain (**1**) may thus be regarded as the biogenetic precursor of vedelianin[3] and a lower homologue of schweinfurthin C,[6] carrying a prenyl group in place of a geranyl group in ring B. It is interesting to note that, although **1** and schweinfurthin C are structurally related, they appear to differ substantially in their biological effects. Mappain is significantly cytotoxic (Table 2), whereas schweinfurthin C is reported to be inactive. The biological differences are maintained in regard to schweinfurthins A and B. These latter two compounds were shown to be particularly effective against cancer cells of CNS origin and were ineffective against ovarian cells,[6] whereas **1** was effective against both an ovarian (SK-OV-3) and a breast cancer (MDA-MB-435) cell line, with $IC_{50}$ values in the low micromolar range (Table 2). We have shown previously that these cell lines are sensitive to other cytotoxic agents.[9,10]

Moreover, unlike many other cytotoxins, **1** appears to be a poor substrate for P-glycoprotein-mediated transport because the parental cell line SK-OV-3 and the multidrug-resistant SKVLB-1 cell line are equally sensitive to mappain (**1**) (Table 2). The SKVLB-1 cell line is multidrug-resistant due to the overexpression of P-glycoprotein.[11] It was derived from the SK-OV-3 cell line by selection with vinblastine and, in our hands, shows a resistance factor of 6400 for vinblastine and of 690 for colchicine.[10] The resistance factor is defined as the $IC_{50}$ value for the drug-resistant line divided by the $IC_{50}$ value for the drug-sensitive parental cell line. Using these two cells lines, we have determined resistance factors for a number of natural products. We obtained values ranging from 1 for **1** and isolaulimalide,[9] indicative of no resistance, to greater than 58 000 for Taxol, suggesting that the latter compound is a substrate for extracellular transport by P-glycoprotein.[9]

## Experimental Section

**General Experimental Procedures.** Optical rotation data were obtained on a JASCO DIP-370 instrument. UV/vis spectra were measured on a Hewlett-Packard model 8453 diode-array spectrophotometer. IR spectra were recorded on a Perkin-Elmer 1600 series FTIR instrument. NMR spectra were recorded on a Varian INOVA WB 400 instrument with a 5-mm Z-gradient probe. MS was performed on a ZAB E instrument in the EI mode. TLC was carried out on Merck Si gel precoated glass plates (0.2 mm thickness), and visualization was performed with phosphomolybdic acid spray or iodine vapor.

**Table 1.** $^1H$ (400 MHz) and $^{13}C$ (100 MHz) NMR Data of **1** in $CDCl_3$.

| position | $\delta_H$ ($J$ in Hz) | $\delta_C$[a] | long-range correlations[b] |
|---|---|---|---|
| 1 | | 141.9 | OH-1, H-3, H-5, H-7 |
| 2 | | 144.4 | OH-2, H-3 |
| 3 | 6.94 d, 1.8 | 110.8 | H-5, H-1′ |
| 4 | | 137.6 | H-5, H-1′ |
| 5 | 6.78 d, 1.8 | 120.6 | H-7, H-1′ |
| 6 | | 127.4 | H-7 |
| 7 | 3.38 d, 7.1 | 30.0 | H-5 |
| 8 | 5.34 m | 121.5 | H-7 |
| 9 | | 139.1 | H-10, H-11 |
| 10 | 1.80 s | 16.2 | |
| 11 | 2.10 m | 39.6 | H-10 |
| 12 | 2.10 m | 26.3 | H-11 |
| 13 | 5.07 m | 123.7 | H-12, H-15, H-16 |
| 14 | | 132.2 | H-12, H-15, H-16 |
| 15 | 1.60 s | 17.7 | H-16 |
| 16 | 1.70 s | 25.7 | H-15 |
| 1′ | 6.85 d, 16.1 | 128.5 | H-3, H-5 |
| 2′ | 6.74 d, 16.1 | 126.2 | H-4′ |
| 3′ | | 130.0 | H-2′ |
| 4′ | 6.53 s | 106.3 | H-1′, H-2′ |
| 5′ | | 155.0 | OH-5′, H-1″ |
| 6′ | | 112.9 | H-4′/8′, OH-5′,-7′, H-1″ |
| 7′ | | 155.0 | OH-7′, H-1″ |
| 8′ | 6.53 s | 106.3 | H-1′, H-2″ |
| 1″ | 3.42 d, 7.1 | 22.5 | |
| 2″ | 5.28 m | 121.4 | H-1″ |
| 3″ | | 135.5 | H-1″, H-4″, H-5″ |
| 4″ | 1.83 s | 17.9 | H-5″ |
| 5″ | 1.76 s | 25.8 | H-4″ |
| OH-1 | 5.48 s | | |
| OH-2 | 5.38 s | | |
| OH-5′ | 5.13 s | | |
| OH-7′ | 5.13 s | | |

[a] From HMQC spectrum. [b] From HMBC spectrum.

**Table 2.** Cytotoxicity of **1** in Drug-Sensitive and Drug-Resistant Cell Lines

| cell lines | $IC_{50}$ value ($\mu$M) ± SD[a] |
|---|---|
| human ovarian cancer SK-OV-3[b] | 1.3 ± 0.1 ($n$ = 4) |
| human ovarian cancer SKVLB[c] | 1.3 ± 0.2 ($n$ = 4) |
| human breast cancer MDA-MB-435[b] | 1.0 ± 0.1 ($n$ = 4) |

[a] $n$ = number of replicates. Four complete experiments were run, and the $IC_{50}$ values calculated for each. Within each experiment either triplicate or quadruplicate replicates were run for each data point. [b] Drug-sensitive cell line. [c] Drug-resistant cell line.

**Plant Material.** *M. mappa* was collected at the Lyon Arboretum, University of Hawaii, Oahu, HI. The leaf material was frozen within 3 h of collection. Voucher specimens (accession number Mooberry 178) were deposited in the Department of Botany herbarium and authenticated by Dr. Will McClatchey.

**Extraction and Isolation.** Crushed, freeze-dried leaf material (100 g from 350 g fresh wt) was extracted twice with 1.2 L $CH_2Cl_2$–*i*-PrOH (7:3) each for 24 h at room temperature. The solids were filtered off, and the filtrate was concentrated to dryness in vacuo below 40 °C. The resulting solid (7 g) was dissolved in 250 mL of MeOH–$H_2O$ (9:1) and extracted with 3 × 250 mL of hexanes. The aqueous MeOH layer was diluted by addition of $H_2O$ (100 mL) and extracted with 3 × 350 mL of $CHCl_3$. The combined $CHCl_3$ extracts were evaporated to dryness (3 g) and dissolved in $CH_2Cl_2$–hexanes (1:1). This

solution was applied to a Si gel column equilibrated in $CH_2Cl_2$–hexanes (1:1). The column was washed with 3 bed volumes each of $CH_2Cl_2$–hexanes (1:1), pure $CH_2Cl_2$, $CH_2Cl_2$–EtOAc (1:1), and EtOAc. The $CH_2Cl_2$–EtOAc (1:1) fraction contained all of the biological activity. The fraction was evaporated to dryness (1.8 g) and chromatographed over Si gel with EtOAc–hexanes (1:2). The active fractions were combined, evaporated to dryness (0.55 g), and dissolved in MeOH. The MeOH solution was applied to a column of Sephadex LH-20 (15 × 950 mm) equilibrated in MeOH and eluted with MeOH. Active fractions were evaporated to dryness (0.2 g) and purified by flash chromatography with methyl–*tert*-butyl ether–hexanes (1:1) to yield **1** (90 mg) as a beige powder.

**Mappain (1):** beige powder; $[\alpha]^{20}_D$ 0° (*c* 0.2, MeOH); UV (MeOH) $\lambda_{max}$ 330 (log $\epsilon$ 3.55) nm; IR (NaCl) $\nu_{max}$ 3415, 2970, 2925, 1620, 1500, 1440, 1300, 1160, 1045, 960, 850 $cm^{-1}$; $^1H$ (400 MHz, $CDCl_3$) and $^{13}C$ NMR data, see Table 1; EIMS *m/z* 449 (14), 448 $[M]^+$(49), 363 (20), 268 (18), 207 (34), 123 (38), 91 (50), 69 (100); HREIMS *m/z* 448.2596 (calcd for $C_{29}H_{36}O_4$, 448.2578).

**Cell Culture and Bioassay.** The SK-OV-3 ovarian cancer cell line (HTB-77) was obtained from American Type Culture Collection (Manassas, VA) and the drug-resistant SKVLB-1 ovarian cancer subline, which was derived from SK-OV-3 cells by selection with vinblastine, was kindly provided by Dr. V. Ling (Vancouver, BC). The MDA-MB-435 cell line was obtained from Dr. M. M. Hijazi (Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC) Cytotoxicity was determined using the sulforhodamine B assay.[12]

**Acknowledgment.** Support of this work by the Department of Defense Breast Cancer Initiative (DAMD17-97-7212) is gratefully acknowledged. We thank Ms. Jennifer Hewett and Mr. Bob Hirano of the Lyon Arboretum for the plant collections; Dr. Will McClatchey of the Department of Botany, University of Hawaii, for taxonomy; and Ms. Georgia Tien for conducting the cytotoxicity assays.

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# Olefin Cross-Metathesis as a Tool in Natural Product Degradation. The Stereochemistry of (+)-Falcarindiol

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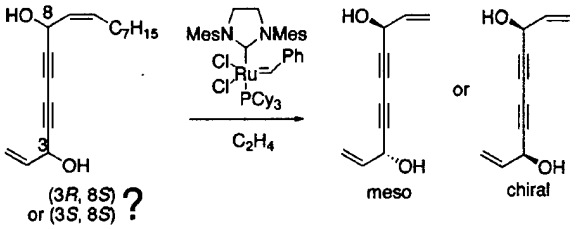
## ABSTRACT




**There are conflicting reports in the literature concerning the absolute sterochemistry at C-3 of the common plant polyacetylene oxylipin (+)-falcarindiol. We have employed olefin cross-metathesis using Grubbs' second generation catalyst and ethylene gas to degrade falcarindiol to the symmetrical 1,9-decadiene-4,6-diyne-3,8-diol. The reaction is completely selective for net removal of the aliphatic side chain. Degradation of (+)-falcarindiol from *Tetraplasandra hawaiiensis* yields a meso product as shown by chiral HPLC. Hence, (+)-falcarindiol from this source has a (3*R*,8*S*)-configuration.**

As part of an ongoing research program on the discovery of new antitumor agents, we examined the organic extract of the Hawaiian endemic plant *Tetraplasandra hawaiiensis*. Bioassay-guided fractionation using a cytotoxicity assay led us to a group of oxylipins of which falcarindiol (+)-**1** was the major constituent.

During our efforts to determine the stereochemistry of the minor oxylipins from this extract we also reviewed the information available regarding (+)-falcarindiol. The absolute configuration of (+)-**1** from *Peucedanum oreoselinum* (entry 1, Table 1) had first been assigned as 3*R*,8*S* by Lemmich in 1981 on the basis of chemical correlation studies.[1] However, in 1996, a group from the NCI proposed a (3*S*,8*S*)-configuration for a similarly strongly dextrorotatory sample of (+)-**1** isolated from *Dendropanax arboreus* (entry 2, Table 1).[2] The assignment of the stereochemistry of the latter sample was based on an application of the advanced Mosher method.[3] It should be noted, however, that the analysis of chemical shift changes in bis-MTPA esters of 1,*n*-diols may be fraught with problems as demonstrated by Riguera.[4] In addition, in the case of (+)-**1**, one has to rely on the analysis of $\Delta\delta$-values from resonances for protons on only one side of the MTPA-plane, a practice that the developers of the method and also Riguera have cautioned against.[3,5]

**Table 1.** Optical Rotations and Assigned Configurations of Falcarindiol

| entry | $[\alpha]_D$ | assigned configuration | ref |
|---|---|---|---|
| 1 | +284[a] | 3*R*,8*S* | ref 1 |
| 2 | +300[b] | 3*S*,8*S* | ref 2 |
| 3 | +219.4[c] | 3*R*,8*S* | refs 6 and 7 |
| 4 | +302[a] | 3*R*,8*S* | this work |
| 5 | +276[b] | 3*R*,8*S* | this work |
| 6 | +250[c] | 3*R*,8*S* | this work |

[a] (*c* 1.0, ether). [b] (*c* 0.14, ether). [c] (*c* 4.6, $CHCl_3$).

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Without reference to the report from the NCI, in 1999 Cai et al. published a chemical synthesis of (+)-(3*R*,8*S*)-falcarindiol.[6] The stereocenters were introduced by transformations of L-tartaric acid and D-xylose. The former gave rise to the C-8 stereochemistry, while the latter was used to generate the absolute configuration at C-3. The synthetic product had an optical rotation and other spectroscopic data reportedly matching those of falcarindiol isolated from *Glehnia littoralis* (entry 3, Table 1).[7]

The sample of **1** that we had isolated from *T. hawaiiensis* showed optical rotations (entries 4–6, Table 1) matching (within experimental error) all of the literature values reported for either of the diastereomers of **1** for which an absolute configuration had been proposed. Hence an unambiguous assignment of the absolute configuration of our sample could not be made on the basis of the chiroptical data available in the literature. We therefore decided to pursue a determination by an independent method. We reasoned that a confirmation of either of the proposed absolute configurations might be obtained if it were possible to modify the C-1/C-2 and C-9/C-10 double bonds present in falcarindiol in such a way that a symmetrical compound is formed. The degradation product would either be meso or chiral if (+)-**1** possesses a (3*R*,8*S*)- or (3*S*,8*S*)-configuration, respectively. However, all attempts to reduce this idea to practice using oxidative methods on TBS-protected **1** failed, as no tractable products could be isolated.[8,9]

At this point we considered the use of olefin cross-metathesis on **1** as an alternative to oxidative degradation (Figure 1).[10]

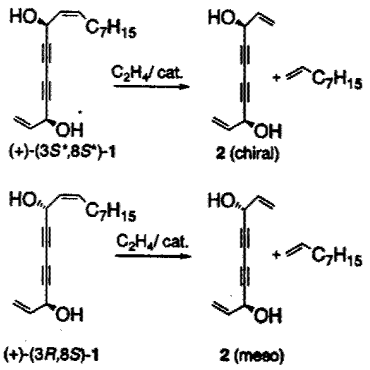
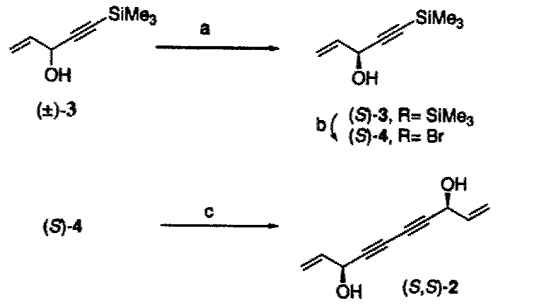



**Figure 1.** Hypothetical stereochemical consequences of the degradation of diasteroisomers of (+)-**1** by cross-metathesis.

In the simplest implementation of this approach, ethylene gas would serve as the second olefin in a reaction catalyzed by one of Grubbs' ruthenium carbenes. A review of the available literature did not reassure us that removal of the C-11 to C-17 chain, the desired reaction, would prevail over potential competing ones such as intermolecular enyne metathesis in the multifunctional environment of **1**.[11] However, side reactions proved to be much less of a problem than we had anticipated. Using the second-generation Grubbs catalyst (10 mol %, DCM, 16 h, room temperature, ethylene-filled double balloon, 1 mM (+)-**1**),[12] we observed a quantitative conversion ($^1$H NMR, TLC) of **1** to a single, slightly more polar compound. The product was isolated in 81% yield by careful chromatography and shown to be **2** (see Supporting Information). We deemed chromatography to be necessary due to the large specific rotation of **1**. Potential contamination of **2** by small amounts of **1** not detectable by $^1$H NMR or TLC might induce a measurable optical rotation in samples of **2** and hence might lead to an erroneous conclusion as to its configuration. In the event, the crystalline sample of **2** isolated from the cross-metathesis reaction possessed marginal optically activity $[\alpha]_D$ +5 (*c* 3.8, $CHCl_3$), which was suspiciously low when compared to that of (+)-**1**.

For comparison purposes, we therefore prepared a synthetic sample of (*S*,*S*)-**2** shown in Scheme 1. Thus, 5-tri-

**Scheme 1.** Synthesis of (*S*,*S*)-1,9-Decadiene-4,6-diyne-3,8-diol[a]




[a] Reaction conditions: (a) lipase from *Pseudomonas fluorescens*, vinyl acetate, 4 Å MS, hexanes, room temperature, 20 h, 35%; (b) NBS, cat. $AgNO_3$, acetone, 3 h, 55%; (c) CuCl, $NH_2OH{\cdot}H_2O$, ethylamine, aqueous MeOH, room temperature, 16 h, 80%.

methylsilylpent-1-en-4-yne-3-ol (±)-**3** was resolved using lipase from *Pseudomonas*.[13] The remaining nonacylated (*S*)-**3**

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($[\alpha]_D$ +38 (*c* 2.0, $CHCl_3$), ≥95% ee) was desilylated and converted to bromide (*S*)-**4** using Isobe's convenient one-pot procedure.[14] Dimerization of (*S*)-**4** under Cadiot–Chodkiewicz conditions yielded (*S,S*)-**2** ($[\alpha]_D$ +102 (*c* 3.8, $CHCl_3$)).

The assignment of an (*S*)-configuration to the nonacylated (+)-enantiomer of **3**, and hence to (+)-**2**, rests on two lines of evidence. First, the results of an application of the advanced Mosher method to (+)-**3** are in accord with this assignment (see Supporting Information).[3] Compound **3** was chosen for the analysis because it bears the TMS group, which yielded one data point on the right side of the MTPA plane. Second, in resolutions of similarly substituted propargylic alcohols, the remaining starting material was shown to have an (*S*)-configuration.[13,15]

The low optical rotation of the sample of **2** from the degradation could be indicative of meso stereochemistry. However, a stereorandom sample of low or null optical activity might also result from epimerization of **1** or of **2** during the cross-metathesis by a variant of the hydride shift mechanism first proposed by Hoye.[16] To investigate this possibility we resorted to analytical HPLC on a chiral stationary phase.

Upon chromatography on a Chiralcel OD column, a sample of symmetrical diol **2** derived from (±)-**4** yielded three well-resolved peaks in a 1:2:1 ratio. This result, albeit not necessarily in that elution order, is to be expected in a successful separation and resolution of a statistical mixture of meso and chiral diastereomers. Synthetic (+)-(*S,S*)-**2** from dimerization of (+)-**4** showed only one peak, which coincided with the last-eluting peak from the stereorandom sample of **2**. Last, **2** obtained from degradation of (+)-**1** from *T. hawaiiensis* gave rise to only one peak, which eluted at the same retention time as the large peak due to the meso diastereomer of **2** in the synthetic sample (see Supporting Information). This result suggests that epimerization does not accompany cross-metathesis when (+)-**1** is being degraded to **2** because the product is the pure meso isomer. It is important to note that the same meso isomer is also found exclusively if the crude degradation reaction mixture is analyzed directly by chiral HPLC without prior purification and crystallization of **2**. In an additional control experiment, (+)-**2** was subjected to the cross-metathesis conditions used for degradation of (+)-**1**. This did not result in any noticeable change in optical purity of (+)-**2** as shown by chiral HPLC analysis of the crude reaction mixture and by polarimetry.

The present results prove unambiguously that (+)-**1** from *T. hawaiiensis* has the same (3*R*,8*S*)-stereochemistry as the material obtained by Cai et al. through total synthesis. Upon cross-metathesis with ethylene, a sample of this configuration is expected to yield the meso isomer of **2**, as is observed experimentally.

Having established the absolute stereochemistry of (+)-**1** from *T. hawaiiensis* unambiguously, we were then able to validate the advanced Mosher method for application in such diyne–diol systems. The result of an analysis of the $\Delta\delta(\delta_S - \delta_R)$ values of the bis-MTPA esters according to the established model was in accordance with (3*R*,8*S*)-stereochemistry of this sample. The resonances for protons H-9, H-10, and H-11 all showed positive $\Delta\delta$ values, while those of the resonances for H-$1_E$, H-$1_Z$, and H-2 were all negative. The sample of (+)-**1** from *D. arboreus* (entry 2, Table 1) had shown all negative $\Delta\delta$ values, which had been interpreted as indicating a (3*S*,8*S*)-configuration.[2] Our results, being clearly different, indirectly support this assignment. Unfortunately, we were not able to obtain a sample of this material for degradation by our method for a rigorous confirmation. However, it appears that Nature does indeed elaborate two diastereomeric forms of (+)-**1**, which cannot be distinguished by polarimetry. Hence, all assignments of stereochemistry to samples of **1** and analogous compounds using this latter method must be regarded as suspect.

In conclusion, we have demonstrated that olefin cross-metathesis using ethylene can be a viable alternative to the classical oxidative degradation procedures for natural products containing double bonds. Olefin metathesis has revolutionized synthetic organic chemistry. This is a consequence of the outstanding functional group tolerance of Grubbs' and Schrock's metathesis catalysts, which are all commercially available.[17,18] Our results suggest that natural product chemists interested in structure elucidation may derive a similar benefit especially from the use of the robust and easy-to-handle Ru-based catalysts.

It is worth noting that our earlier attempts to perform the metathesis reaction on TBS-protected **1** were unsuccessful. Furthermore, in the present circumstance, the use of ethylene as the donor olefin proved to be preferable over the more nucleophilic allyltrimethylsilane because the latter yielded an inseparable mixture of olefin geometrical isomers.[19]

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**Supporting Information Available:** Experimental procedures for the preparation of (+)-**2**, $^1H$ NMR data for configuration analysis and optical purity determination of (+)-**3**, chiral HPLC chromatograms of **2**, Mosher analysis of (+)-**1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting information

# Olefin Cross-metathesis as a Tool in Natural Products Degradation. The Stereochemistry of (+)-Falcarindiol.

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(±)-5-Trimethylsilylpent-1-en-4-yne-3-ol **3**.

To a solution of (trimethylsilyl)acetylene (1.04 g, 1.5 mL, 10.6 mmol) in dry THF (42 mL) at –78 °C was added dropwise a solution of n-butyllithium (2.4 M in hexanes, 4.42 mL, 10.6 mmol) under a $N_2$ atmosphere. After the addition was complete, the solution was allowed to warm to –60 °C over a period of 15-30 min, at which point an ice-cold solution of acrolein (0.71 g, 0.85 mL, 12.7 mmol) in THF (25 mL) was introduced slowly *via* a cannula. The resulting mixture was allowed to gradually warm to 0 °C over a period of 1.5 h. After stirring for an additional 1 h at room temperature, the mixture was quenched with cold satd. $NH_4Cl$. The aqueous phase was extracted with $Et_2O$ (×3), dried ($MgSO_4$) and concentrated to dryness in *vacuo*. The resulting crude oil was purified by Kugelrohr distillation (bp 85-90 °C/20 mmHg) to give 1.63 g (95% yield) of pure racemic allyl alcohol (±)-**3** as a colorless liquid. FTIR (film) 3355 (br), 2175, 1640, 1405, 1250, 1115, 1030, 985, 845, 760 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 5.94 (ddd, J= 17.1, 10.2, 5.4 Hz, 1H), 5.44 (dd, J= 17.1, 0.9 Hz, 1H), 5.19 (dd, J= 10.2, 0.9 Hz, 1H), 4.85 (brd, J= 5.4 Hz, 1H), 2.60 (brs, 1H), 0.16 (s, 9H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 136.9, 116.7, 104.3, 91.2, 63.6, -0.03 (3C).

(+)-5-Trimethylsilylpent-1-en-4-yne-3-ol (*S*)-**3**

To a well-stirred suspension of lipase (*Pseudomonas fluorescens*, 0.03 g, 0.15 mass equiv), ground activated 4-Å molecular sieves (0.22 g, 1.0 mass equiv) and racemic allyl alcohol (±)-**3** (0.22 g, 1.45 mmol) in dry hexane (12 mL) was added vinyl acetate (0.53 mL, 5.8 mmol) in one portion. The suspension was allowed to stir under $N_2$, at room temperature for 21 h, and the course of the reaction was monitored by $^1H$ NMR analysis. At the end of this period, the mixture was filtered through a pad of Celite, and evaporated to dryness under reduced pressure. The crude product mixture following flash chromatography gave (*R*)-acetate (eluent: 3% $Et_2O$/hexane) 0.20 g (35% yield, ee > 98%) and alcohol (*S*)-**3** ((eluent: 10% $Et_2O$/hexane) 0.16 g (35% yield, ee > 98%) as colorless oils: (*S*)-**3** ≥95 % ee; $(\alpha)_D^{23}$ 38 (c 2.0, $CHCl_3$); for spectroscopic data see (±)-**3**.

(+)-5-bromopent-1-en-4-yne-3-ol (*S*)-**4**.

A solution of alcohol (*S*)-**3** (0.05 g, 0.32 mmol) in acetone (2 mL) was treated with N-bromosuccinimide (0.07 g, 0.39 mmol) and catalytic powdered $AgNO_3$ (0.004 g, 0.02 mmol). The reaction mixture was wrapped with aluminum foil to exclude light and stirred at room temperature for 3 h. The mixture was diluted with cold water and extracted with $Et_2O$ (×3). The combined organic layers were dried ($MgSO_4$), concentrated to dryness under *vacuo* and subjected to flash column chromatography (silica gel, 10% $Et_2O$-hexane) to afford 0.03 g (55% yield) of bromo acetylene (*S*)-**4** as a clear liquid: $(\alpha)_D^{25}$ 38 (c 1.9, $CHCl_3$); FTIR (film) 3380 (br), 2215, 1640, 1405, 1265, 1120, 1015, 985, 935, 885, 800, 725 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 5.95 (ddd, J= 17.1, 9.9, 5.4 Hz, 1H), 5.46 (ddd, J= 17.1, 0.9, 0.9 Hz, 1H), 5.25 (ddd, J= 9.9, 0.9, 0.9 Hz, 1H), 4.90 (ddd, J= 6.3, 5.4, 0.9 Hz, 1H), 2.15 (d, J= 6.3 Hz, 1H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 136.4, 117.2, 79.0, 64.2, 47.2.

(*S,S*)-1,9-decadiene-4,6-diyne-3,8-diol **2**.

To an ice-cold solution of 70% aqueous $EtNH_2$ (0.060 mL) and catalytic CuCl (0.002 g, 0.02 mmol) in MeOH (0.074 mL, degassed with Ar) was added $NH_2OH.HCl$ (0.007 g, 0.11 mmol) dissolved in water (0.026 mL). To the above mixture at 0 °C was added a solution of bromo

acetylene (*S*)-**4** (0.052 g, 0.32 mmol) in MeOH (0.65 mL). After the addition was complete, the reaction mixture was removed from the ice-bath and stirred at room temperature overnight. The solution was diluted with cold water and extracted with $Et_2O$. The organic layers were dried ($MgSO_4$), concentrated under reduced pressure and the resulting crude residue was purified on silica gel (20%-30% EtOAc-hexane) to yield 0.011 g (40% yield) of symmetrical dimer (*S,S*)-**2** as a white crystalline solid: $[\alpha]_D^{24}$ 102 (c 0.38, $CHCl_3$); mp 77-78 °C; FTIR (film) 3320 (br), 2355, 2145, 1635, 1405, 1260, 1115, 1015, 985, 935, 865 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) δ 5.95 (ddd, J= 17.1, 10.2, 5.4 Hz, 2H), 5.48 (ddd, J= 17.1, 0.9, 0.9 Hz, 2H), 5.27 (ddd, J= 10.2, 0.9, 0.9 Hz, 2H), 4.95 (brm, 2H), 2.03 (d, J= 4.8 Hz, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$) δ 135.9 (2C), 117.7 (2C), 78.6 (2C), 70.3 (2C), 63.7 (2C).

Degradation of (+)-**1** to **2**.

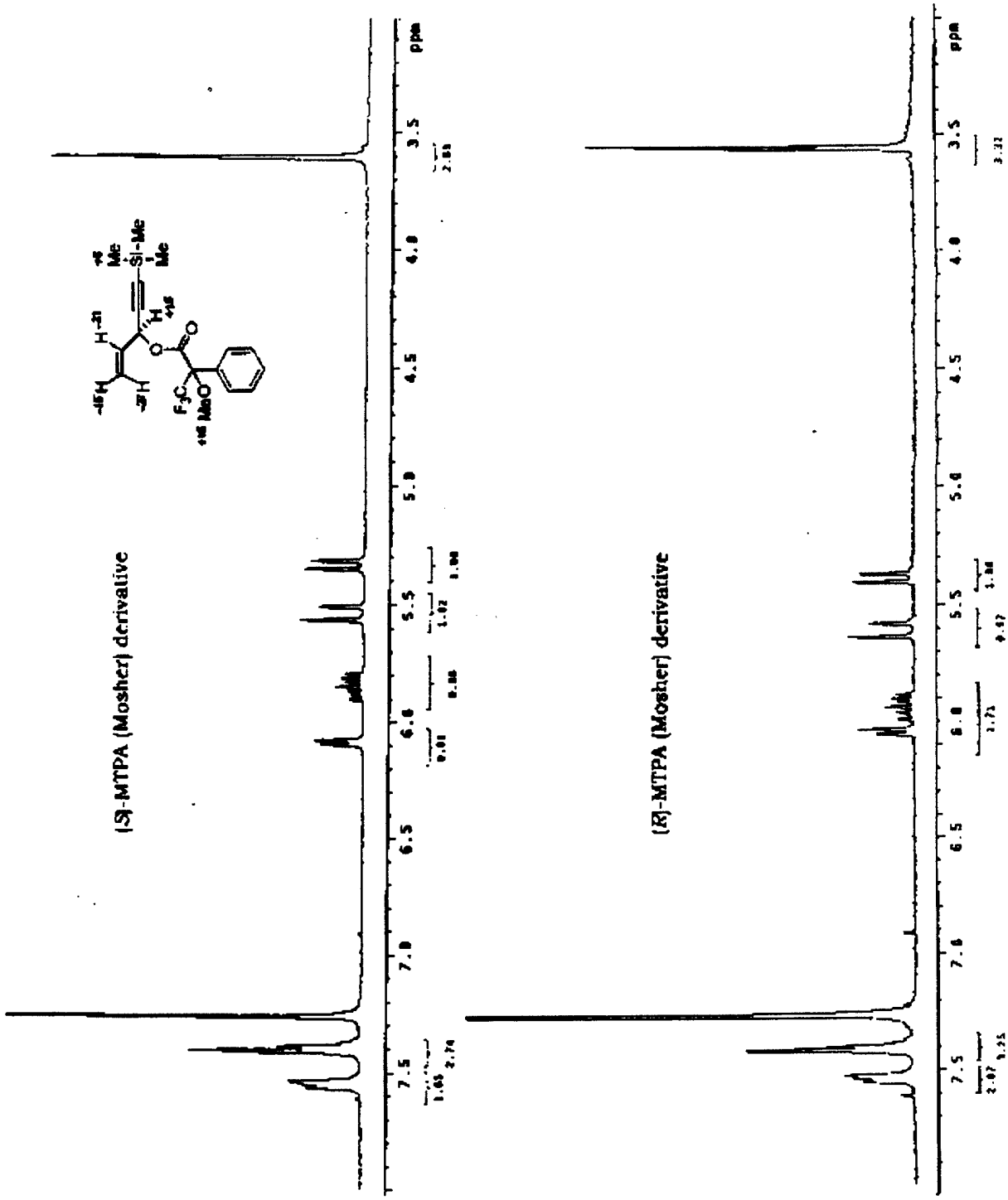
A solution of second-generation Grubbs' catalyst (0.011 g, 0.013 mmol, 10 mol%) in degassed anhydrous $CH_2Cl_2$ (45.5 mL) at room temperature was stirred vigorously under an atmosphere of ethylene gas. After bubbling a steady stream of ethylene gas for 15 min, a solution of (+)-**1** (0.035 g, 0.13 mmol) in degassed $CH_2Cl_2$ (70 mL) under ethylene gas was added slowly *via* a cannula to the above reaction flask at room temperature. Progress of the reaction was monitored by TLC analysis. After stirring overnight (16 h), the solvent was removed in *vacuo* and the residue was purified by flash column chromatography (silica gel, 20-30% EtOAc-hexane) to afford the symmetrical dimer **2**-(meso) as a slightly brown solid. The crude solid was re-crystallized in $Et_2O$-hexane to give 0.013 g (81% yield) of pure product as white crystalline material: $[\alpha]_D^{23}$ 5 (c 0.7, $CHCl_3$); mp 49-51 °C; FTIR (film) 3300 (br), 2360, 2145, 1640, 1400, 1325, 1260, 1115, 1015, 985, 930, 865 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 5.94 (ddd, J= 17.0, 10.1, 5.4 Hz, 2H), 5.48 (d, J= 17.0 Hz, 2H), 5.27 (d, J= 10.1 Hz, 2H), 4.95 (d, J= 5.4 Hz, 2H), 2.36 (brs, 2H); $^{13}C$ NMR (125 MHz, $CDCl_3$) δ 135.6 (2C), 117.5 (2C), 78.3 (2C), 70.1 (2C), 63.4 (2C).

General procedure for the preparation of MTPA-esters:

A 0.01 M solution of alcohol product in anhydrous $CH_2Cl_2$ at 0 °C was treated successively with 2,6-lutidine (8 mol equiv), MTPA-Cl (4 mol equiv per OH) and DMAP (1 mol equiv per OH) under $N_2$. The mixture was allowed to stir at room temperature for 15 h and treated with 3(N,N-dimethylamino)propylamine (0.3 mol equiv over acid chloride). After stirring for an additional 10 min, the solvent was removed under *vacuo*. The residue was dissolved in a minimum amount of $CH_2Cl_2$ and applied to a silica gel column (5% $Et_2O$-hexane) to afford pure ester in 85-90% yield:

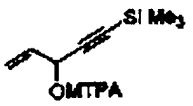
(+)-(3*R*,8*S*)-**1**-(*S*)-MTPA-*bis*-ester: $^1H$ NMR (500 MHz, $CDCl_3$) 7.52-7.50 (m, 5H), 7.41-7.38 (m, 5H), 6.40 (d, J= 9.0 Hz, 1H), 6.09 (d, J= 5.4 Hz, 1H), 5.92 (m, 1H), 5.73 (brm, 1H), 5.60 (d, J= 16.5 Hz, 1H), 5.46 (m, 1H), 5.43 (d, J= 10.5 Hz, 1H), 3.63 (s, 3H), 3.58 (s, 3H), 2.15 (m, 2H), 1.45-1.17 (m, 10H), 0.88 (t, J= 6.9 Hz, 1H). (+)-(3*R*,8*S*)-**1**-(*R*)-MTPA-*bis*-ester: $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.52-7.49 (m, 5H), 7.41-7.39 (m, 5H), 6.35 (d, J= 8.6 Hz, 1H), 6.11 (d, J= 5.8 Hz, 1H), 5.82 (ddd, J= 16.9, 10.2, 5.8 Hz, 1H), 5.77 (m, 1H), 5.55 (m, 1H), 5.52 (d, J= 16.9 Hz,

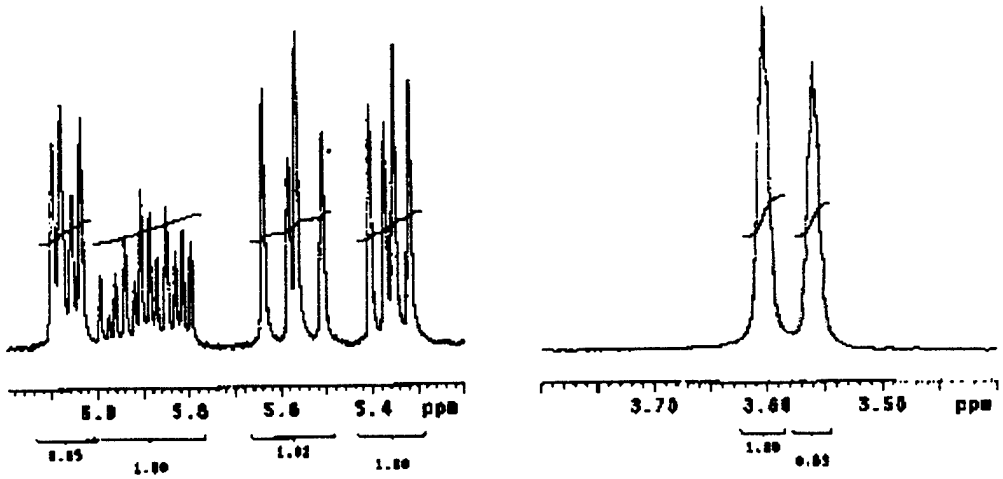
1H), 5.37 (d, J= 10.2 Hz, 1H), 3.60 (s, 3H), 3.57 (s, 3H), 2.17 (m, 2H), 1.40-1.24 (m, 10H), 0.87 (t, J= 7.1 Hz, 1H).



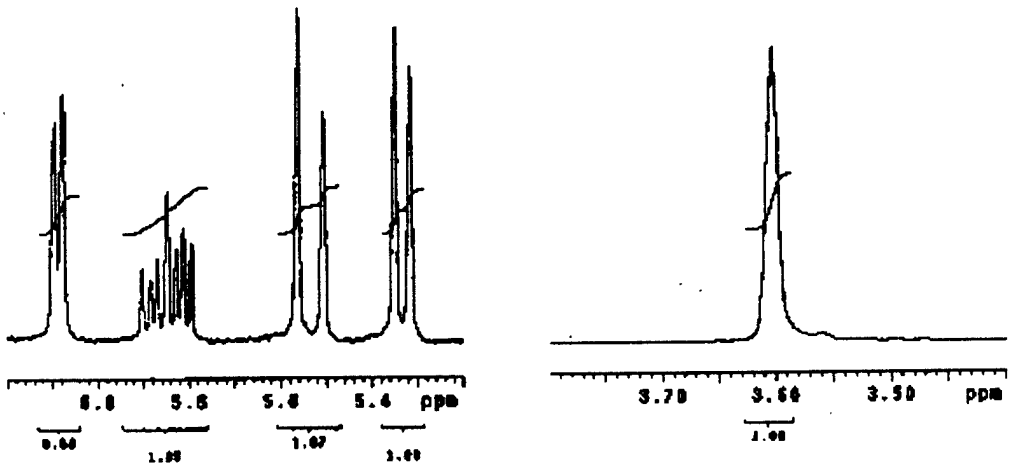

Δδ Values (Δδ = $\delta_S - \delta_R$) measured in Hertz (300 MHz, $CDCl_3$).

(±)-3-(*S*)-MTPA ester



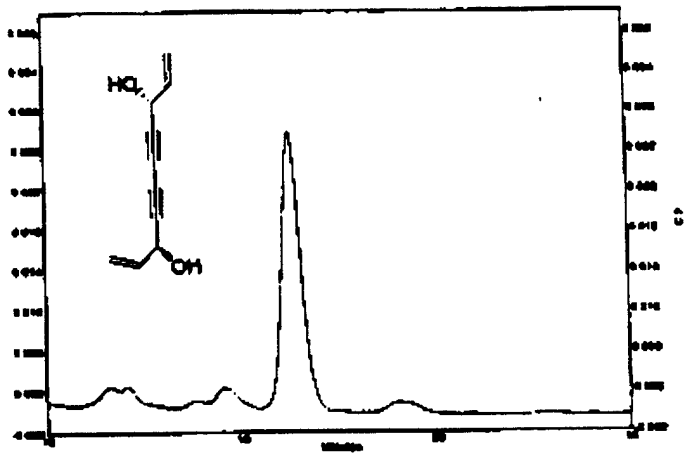




(+)-3-(*S*)-MTPA ester

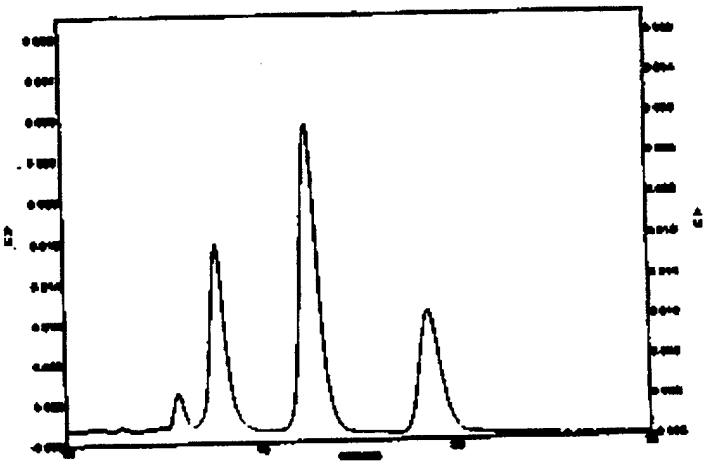




(*S*)-MTPA (Mosher) derivatives of **3** (300 MHz, $CDCl_3$).

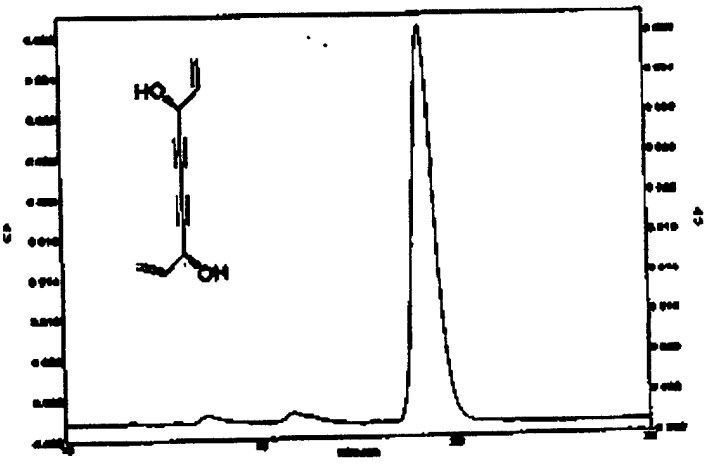
**2** (meso)
retention time (min)- 16.28 (*R*,*S*)




Synthetic (±)-**2** (racemic)
retention times (min)- 13.90 (*R*,*R*)
16.27 (*R*,*S*)
19.33 (*S*,*S*)



Synthetic (+)-**2** (chiral)
retention time (min)- 19.22 (*S*,*S*)




HPLC (chiral) Chiralcel OD (250×4.6 mm), 23 °C, λ=254 nm,
15% iPrOH-hexane, 1 mL/min

**Table 10.** $^{13}C$ and $^{1}H$-NMR Data of **28** and Cucurbitacin E in chloroform-d

| Atom No. | 28 | | Cucurbitacin E[25] | |
|---|---|---|---|---|
| | $^{13}C$ δ (ppm) | $^{1}H$ δ (ppm) | $^{13}C$ δ (ppm) | $^{1}H$ δ (ppm) |
| 1 | 114.8 | 5.94 (1H, d, $J$ = 2.8 Hz) | 115.2 | 5.93 (1H, d, $J$ = 2 Hz) |
| 2 | 144.5 | | 144.7 | |
| 3 | 198.7 | | 198.8 | |
| 4 | 47.5 | | 47.5 | |
| 5 | 136.7 | | 136.5 | |
| 6 | 120.8 | 5.76 (1H, m) | 120.5 | 5.76 (1H, br. s) |
| 7 | 23.5 | 2.02 (1H, m) | 23.5 | 2.10 (1H, m) |
| | | 2.37 (1H, ddt, $J$ = −19.8, 2.8, 2.7 Hz) | | 2.43 (1H, m) |
| 8 | 41.6 | 2.02 (1H, m) | 41.5 | 2.05 (1H, d, $J$ = 8 Hz) |
| 9 | 48.8 | | 48.8 | |
| 10 | 34.6 | 3.50 (1H, br. s) | 34.6 | 3.51 (1H, br. s) |
| 11 | 212.8 | | 213.0 | |
| 12 | 48.8 | 2.71 (1H, d, $J$ = −14.4 Hz) | 48.6 | 2.70 (1H, d, $J$ = −14 Hz) |
| | | 3.21 (1H, d, $J$ = −14.4 Hz) | | 3.21 (1H, d, $J$ = −14 Hz) |
| 13 | 50.7 | | 48.8 | |
| 14 | 48.1 | | 50.5 | |
| 15 | 45.5 | 1.46 (1H, d, $J$ = −13.3 Hz) | 45.2 | 1.70 |
| | | 1.88 (1H, dd, $J$ = −13.3, 7.9 Hz); | | 1.95 |
| 16 | 71.2 | 4.37 (1H, t, $J$ = 7.9 Hz) | 70.7 | 4.39 (1H, t, $J$ = 7 Hz) |
| 17 | 58.2 | 2.48 (1H, d, $J$ = 7.9 Hz) | 57.9 | 2.49 (1H, d, $J$ = 7 Hz) |
| 18 | 19.8 | 0.98 (3H, s) | 19.8 | 0.98 |
| 19 | 20.0 | 1.02 (3H, s) | 17.5 | 1.41 |
| 20 | 78.2 | | 78.6 | |
| 21 | 23.9 | 1.43 (3H, s) | 23.7 | 1.34 |
| 22 | 202.5 | | 202.4 | |
| 23 | 120.3 | 6.46 (1H, d, $J$ = 15.6 Hz) | 120.7 | 6.46 (1H, d, $J$ = 15 Hz) |
| 24 | 151.9 | 7.05 (1H, d, $J$ = 15.6 Hz) | 151.7 | 7.04 (1H, d, $J$ = 15 Hz) |
| 25 | 79.3 | | 79.3 | |
| 26 | 26.4 | 1.53 (3H, s) | 26.0 | 1.55 |
| 27 | 25.9 | 1.56 (3H, s) | 26.1 | 1.56 |
| 28 | 18.3 | 1.38 (3H, s) | 19.8 | 1.38 |
| 29 | 20.2 | 1.35 (3H, s) | 20.0 | 1.26 |
| 30 | 27.9 | 1.24 (3H, s) | 27.7 | 1.02 |
| 31 | 170.0 | | 170.1 | |
| 32 | 21.9 | 2.00 (3H, s) | 21.8 | 2.00 |
| 33 | | 4.26 (1H, s) | | |
| 34 | | 1.70-1.90 | | |
| 35 | | 5.92 | | |

$^{1}H$ and $^{13}C$-NMR spectra of **28** were measured at 500MHz and 125MHz, respectively. $^{1}H$ and $^{13}C$-NMR spectra of cucurbitacin E were reported at 360MHz and 90MHz, respectively.

Select $^{1}H$ NMR and $^{13}C$ NMR data for crithmumdiol **5** from *Tetraplasandra hawaiiensis*.

| Position # | $^{13}C$ NMR (δ, ppm) | $^{1}H$ NMR(δ, ppm) (multiplicity, coupling constant) |
|---|---|---|
| 1 | 116.2 | 5.29 (1H, ddd, *J*= 1.2/1.1/10.4 Hz) |
| | | 5.19 (1H, ddd, *J*= 1.1/1.1/16.5 hz) |
| 2 | 138.1 | 5.85 (1H, ddd, *J*= 5.9/10.4/16.5 Hz) |
| 3 | 73.1 | 4.68 (1H, ddd, *J*= 1.1/5.8/ 5.9 Hz) |
| 4 | 143.8 | 6.15 (1H, ddd, *J*= 1.1/5.8/16.0 Hz) |
| 5 | 109.8 | 5.78 (1H, ddd, *J*= 1.6/1.6/16.0 Hz) |
| 6 | 82.6 | - |
| 7 | 90.3 | - |
| 8 | 58.6 | 5.27 (1H, m) |
| 9 | 128.7 | 5.25 (1H, m) |
| 10 | 133.8 | 5.59 (1H, m) |
| 11 | 27.6 | 2.09-2.149 (2H,m) |
| 12 | 29.1* | 1.35 (2H, m) |
| 13 | 29.2* | 1.26-1.31 |
| 14 | 29.3* | 1.26-1.31 |
| 15 | 31.8 | 1.26-1.31 |
| 16 | 22.6 | 1.26-1.31 |
| 17 | 14.1 | 0.87 (3H, t, *J*=7.3 Hz) |

* assignments are interchangeable

$^{1}H$ NMR data at 500 MHz, $^{13}C$ NMR data at 125 MHz. Data acquired in $CDCl_3$.

Select $^{1}H$ and $^{13}C$ NMR data for compound **6** from *Tetraplasandra hawaiiensis*.

| Position # | $^{13}C$ NMR (δ, ppm) | $^{1}H$ NMR(δ, ppm) (multiplicity, coupling constant) |
|---|---|---|
| 1 | 117.5 | 5.45 (1H, *br*d, *J*= 16.9 Hz) |
| | | 5.28 (1H, *br*d, *J*= 10.3 Hz) |
| 2 | 135.7 | 5.94 (1H, ddd, *J*= 5.4/10.3/16.9 Hz) |
| 3 | 63.5 | 4.95 (1H,*br*d, J= 5.4 Hz) |
| 4 | 78.5 | - |
| 5 | 70.2 | - |
| 6 | 70.1 | - |
| 7 | 78.0 | - |
| 8 | 60.8 | 4.37 (1H, *br*d, *J*= 7.3 Hz) |
| 9 | 57.9 | 3.14 (1H, dd, *J*= 7.32/ 4.05 Hz) |
| 10 | 58.0 | 3.05 (1H, m, *J*= 4.05 Hz) |
| 11 | 27.5 | 2.11 (1H, m) |
| | | 1.63 (1H, m) |
| 12 | 29.3 | 1.2-1.4 |
| 13 | 29.4 | 1.2-1.4 |
| 14 | 29.1 | 1.2-1.4 |
| 15 | 31.7 | 1.2-1.4 |
| 16 | 22.5 | 1.2-1.4 |
| 17 | 14.1 | 0.88 (3H, t, 7.3) |

$^{1}H$ NMR at 500 MHz, $^{13}C$ NMR at 125 MHz. Data acquired in $CDCl_3$.